REMARKS

Claims 1-8, 10, 11, 20-39, 41-43, 45-51, 53, 55, and 57-60 were pending prior to this Response with claims 5, 6, 46-51, 53, 55, and 57-60 having been withdrawn. By the present communication, no new claims have been added or canceled and claims 1-4, 8, 20, 21, 23, 26, 28, 29, 33-35, 37, 43, and 45 have been amended to define Applicants' invention with greater particularity. Support for the amended claims may be found throughout the specification and claims as originally filed. Accordingly, upon entry of the present amendment, claims 1-8, 10, 11, 20-39, 41-43, 45-51, 53, 55, and 57-60 will be pending and under consideration in this application.

Objection to the Claims

Claims 2-4, 21, 33-35, 37, 43, and 45 have been objected to on the basis of allegedly containing various informalities. Without acquiescing to the substantive basis for the objections provided by the Office Action, and in order to expedite prosecution of the instant application, Applicants have amended claims 2-4, 21, 33-35, 37, and 43 as suggested by the Examiner. With regard to claim 45, the Office Action asserts that the phrase "sequences that hybridize under stringent conditions thereto" in step c) should be deleted. Applicants respectfully traverse the objection to this claim and point to page 12, line 28 bridging to page 13, line 2 of the specification as filed, which states "contacting the genomic DNA, or a fragment thereof, comprising one or more sequences selected from the group consisting of SEQ ID NOS: 1 to SEQ ID NO: 64 or a sequence that hybridizes under stringent conditions thereto, with one or more methylation-sensitive restriction enzymes." Applicants submit that the term "sequence that hybridizes under stringent conditions thereto" is not an informality as it refers to a sequence that is complementary to the at least 16 contiguous nucleotides of SEQ ID NO: 5, which may be contacted by methylation-sensitive restriction enzymes. Accordingly, reconsideration and withdrawal of the objections are respectfully requested.

Rejection under 35 U.S.C. §112, 1st Paragraph

Applicants respectfully traverse the rejection of claims 3, 4, and 7 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement.

The Office Action appears to take issue with the phrase "reduced expression of the ALX4 gene (SEQ ID: 5) in the sample as compared with the sample from a subject not having a colon cell proliferative disorder indicative of a colon cell proliferative disorder" in independent claim 3 and alleges the specification does not provide adequate written description support.

Without acquiescing to the rationale offered by the Office Action, and in order to advance prosecution, Applicants have amended independent claim 3. Applicants submit that the specification provides ample support for claim 3 under the provisions of 35 U.S.C. §112, first paragraph, which recites a method for the detection of a colon cell proliferative disorder in a human subject comprising "comparing ALX 4 gene (SEQ ID NO:5) expression level in the sample with ALX 4 gene (SEQ ID NO: 5) expression level of a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells from a human subject not having a colon cell proliferative disorder, wherein reduced expression of the ALX 4 gene (SEQ ID NO:5) in the sample as compared with the sample from the subject not having a colon cell proliferative disorder-is associable with the development of colorectal carcinoma and other colorectal cell proliferative disorder in the subject." Support for the amendments to claim 3 may be found in the specification as originally filed e.g., at page 28, line 11 through page 29, line 21; page 30, line 5 through page 31, line 18; and Example 12 on pages 186-187.

Applicants point to the originally filed specification at e.g, page 28, lines 11-26, which provides guidance to the skilled artisan for the use of AXL4 gene as a marker for the detection of colon cell proliferative disorder and in particular "by any means of the expression of the gene" (lines 16-17). Page 29, lines 1-29 and Example 12 on pages 186-187 of the specification as filed describe in detail a method to detect the presence of mRNA encoding a gene in a detection system for colon cancer. The instant specification provides support for the detection of a colon cell proliferative disorder by analysis of polypeptide

expression encoded by the genes, genomic sequences, or genes regulated by the genomic sequences e.g., at pages 30, line 5 bridging to page 31, line 18.

The specification also provides data for differentially expressed probe sets between subjects not having a colon cell proliferative disorder and subjects having a colon cell proliferative disorder on Table 5 on page 187 and states "[g]enes with a fold change bigger than 2 (or smaller than 0.5) were identified as differentially expressed" thereby fully disclosing to one of ordinary skill in the art how to practice the presently claimed invention.

Applicants further submit that the Federal Circuit has held with regard to the written description requirement

> A claim will not be invalidated on section 112 grounds simply because the embodiments of the specification do not contain examples explicitly covering the full scope of the claim language. That is because the patent specification is written for a person of skill in the art, and such a person comes to the patent with the knowledge of what has come before. Placed in that context, it is unnecessary to spell out every detail of the invention in the specification; only enough must be included to convince a person of skill in the art that the inventor possessed the invention and to enable such a person to make and use the invention without undue experimentation. [79 USPQ2d at 1007 (quoting *LizardTech, Inc., v. Earth Res. Mapping, Inc.*, 424 F.3d 1336, 1345, 76 USPQ2d 1724 (Fed. Cir. 2005))].

After reading the specification, a person of ordinary skill in the art would readily understand how to make and use the invention i.e., detect colon cell proliferative disorder by analysis of AXL4 gene expression in a biological sample taken from a human subject and would recognize that Applicants were in possession of the invention as claimed at the time the application was filed.

In view of the amendments and the foregoing discussion, Applicants respectfully request reconsideration and withdrawal of the rejection as it applies to independent claim 3 and claims dependent therefrom.

Applicants respectfully traverse the rejection of claims 1-4, 7, 8, 10, 11, 20-39, 41-43, 45, and 60 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement.

In particular, the Office Action alleges repeatedly with regard to the comparing step (page 10) that the independent claims 1, 8, 20, and 45 do not "limit the CpG methylation status to the CpG methylation status of ALX4 gene and that the claims are drawn to "any kind of gene from any kind of subject not having a colon cell proliferative disorder." The Office Action further alleges (page 11) the above claims do not require that "the CpG methylation status from the subject not having a colon cell proliferative disorder is generated from genomic DNA of blood plasma, blood serum, whole blood, or isolated blood cells." Based on the foregoing, the Action concludes that it is unclear how to perform the methods of the present claims.

Without acquiescing to the reasoning set forth by the Office Action, and in order to advance prosecution, independent claims 1, 8, 20, and 45 have been amended to recite "comparing the CpG methylation status of ALX4 gene in the sample of a) with the CpG methylation status of ALX4 gene of a biological sample comprising genomic DNA from blood plasma, blood serum, from blood plasma, blood serum, whole blood, or colon cells from a human subject not having a colon cell proliferative disorder, wherein a difference between the CpG methylation status of ALX4 gene from the sample of a) and the CpG methylation status of ALX4 gene from the biological sample from a human subject not having a colon cell proliferative disorder is associable with the development of colorectal carcinoma and other colorectal cell proliferative disorder in the subject."

The Office Action alleges (page 9) generally that "the specification does not provide guidance for the methods recited in claims 1-4, 7, 8, 10, 11, 20-39, 41-43, and 45. Furthermore there is no experimental condition and/or experimental data in the specification to support the claimed invention recited in claims 1-4, 7, 8, 10, 11, 20-39, 41-43, and 45." Applicants respectfully disagree.

It is well known that to satisfy the enablement requirement of 35 U.S.C. §112, first paragraph, the specification must teach one of skill in the art to make and use the invention without *undue* experimentation. *See, Atlas Powder Co. v. E.I. DuPont de Nemours*, 750 F.2d

1569,224 USPQ 409 (1984). This sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything within the scope of a broad claim." *In re Anderson,* 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of §112, first paragraph "can be fulfilled by the use of illustrative examples or by broad terminology." *In re Marzocci* et al., 469 USPQ 367 (CCPA 1971). Moreover, as explained in *In re Angstadt*, 537 F.2d 498, 190 USPQ 214 (CCPA 1976), some experimentation is acceptable. MPEP §2164.01 explains, "[t]he fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." No showing has been made that the work required, as well as any experimentation that may occur along the way, would be anything but routine. In summary, MPEP §2164.01(c) states that "[i]f a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 U.S.C. §112 is satisfied."

Applicants submit that the instant specification provides extensive guidance and numerous working examples for the methods of the present claims and point to page 50, line 14 bridging to page 52, line 2 of the originally filed specification, which describes obtaining a biological sample, isolating the genomic DNA therefrom, converting cytosine bases methylated at the 5′-position to a base possessing dissimilar hybridization characteristics ("treatment" or "pre-treatment"), and amplification of the treatment fragments with primers, which are disclosed, for example, at pages 52 through 74. Detection of the amplificates is disclosed at page 77, line 6 bridging to page 78, line 9 and analysis of the amplificates to ascertain the methylation status of one or more positions according to SEQ ID NOs: 1 to 64 is described at e.g, page 78, line 10 bridging to page 79, line 29 and page 148, lines 3-19. In particular, the specification discloses at page 148, lines 3-19, for SEQ ID NO: 5, the sequences for the oligonucleotides to be used for combined Blocking oligonucleotide-RealTime analysis: Lightcycler dual probe detection assays. Moreover, the specification discloses in detail a variety for conducting the detection method of the present claims e.g., amplification by blocking oligonucleotides or by the MSP technique.

The specification describes at page 161, line 12 bridging to page 165, line 22, the precise experimental conditions and statistical data analysis protocols used to practice the presently claimed methods.

Furthermore, Applicants have presented experimental results beginning on page 165, line 25, which demonstrate the viability of the experimental and statistical procedures discussed above. In the results section, Applicants describe data obtained from comparison of biological samples taken from human subjects with colon cell proliferative disorders versus various biological samples taken from human subjects. For instance, page 167, lines 5-16 describes samples from normal colon human subjects compared to biological samples from human subjects with colon polyps and inflammatory disorders and indicate SEQ ID NO: 5 as being an important marker with statistical significance. At page 167, lines 19-29, the specification discloses experimental results from biological samples taken from "other tissues" of non-colorectal origin in human subjects e.g., peripheral blood lymphocytes compared to those with colorectal cancer stating "[t]hese markers [] enable the detection of colorectal carcinoma cells in, for example, body fluids such as serum." Applicants show on page 168, lines 5-20 experimental date obtained from analysis of methylation status of biological samples from human subjects not having colon cell proliferative disorder and those from non-colorectal sources compared with colorectal carcinoma samples and indicates that SEQ ID NO: 5 (and complements thereof) is a statistically significant marker for colon cancer. At page 169, lines 5-17, the specification describes experimental results from methylation state analysis of biological samples from human subjects not having colon cell proliferative disorder compared with those having colon cell proliferative disorder and indicate that SEQ ID NO: 5 and complements thereof is a significant marker of the disorder.

Because Applicants have repeatedly presented results positively demonstrating a statistically significant differential (low incidence of false positives) in methylation status between non-colon cell proliferative disorder and known colon cell proliferative disorder samples, it is inherent that the specification is enabling with regard to 1) distinguishing between age-related methylation and methylation associated with colon cell proliferative disorder; and 2) distinguishing between colon cell proliferative disorder and other disorders e.g., breast cancer.

Since Applicants have consistently shown that there is a statistically significant differential in methylation status between non-colon cell proliferative disorder in non-colorectal samples and known colon cell proliferative disorder in colorectal samples, it is inherent that the specification is enabling with regard to discriminating between colon cell proliferative disorder and other disorders e.g., adenocarcinomas of the esophagus when the sample is obtained from sources other than colorectal biological samples.

With regard to the Office Action's assertion that claim 21 allegedly does not indicate how to differentiate between (an amplificate amplified from) methylated genomic DNA and (an amplificate amplified from) unmethylated genomic DNA, Applicants respectfully disagree. Differentiation of methylation status is an art-recognized procedure and is fully described in the specification at e.g., page 22, line 29 through page 26, line 11. For instance, lines 20-22 on page 25 of the specification, as filed, state: "MSP (methylation-specific PCR) allows for assessing the methylation status of virtually any group of CpG sites within a CpG island, independent of the use of methylation-sensitive restriction enzymes." Therefore, the skilled artisan would be enabled to make and use the method of claim 21 based on general knowledge in their possession and that disclosed in the specification.

The Office Action asserts that (page 14) "since claims 31, 41, and 42 require hybridizing at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID NOs: 312, 313, 428 and 429, if the nucleic acid molecule is "TTTTTTTTTT" from SEQ ID NOS: 313 and 429 which can hybridize polyA sequence of many different mRNAs, a specific hybridization complex for human ALX 4 gene cannot be formed." Applicants respectfully submit that amplification of bisulfite converted DNA i.e., SEQ ID NOS: 313 and 429 is performed "using primers specific for the CpG islands of interest" (see page 23, lines 13-16) thus, a skilled artisan would not select a primer with the sequence "TTTTTTTTTT" for this purpose. Moreover, CpG sites are regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide in the linear

sequence of bases along its length. Claims 31, 41, and 42 all depend from claim 21, which in turn depends from claim 8, wherein claim 8 includes the following limitation with respect to the 9 contiguous nucleotide segment: "and the contiguous nucleotides comprise at least one CpG dinucleotide sequence." Thus, it is axiomatic that a nucleic acid or primer that would hybridize a 9 contiguous nucleic acid segment of interest of SEQ ID NOS: 313 and 429 would not be "TTTTTTTTTT."

The Office Action contends (page 14) that claim 20 allegedly does not correlate steps a) to c) with detecting colon cell proliferative disorder. The Office Action further asserts (page 15) the phrase "the genomic DNA of b)" in claim 45 may be from any other gene that is not AXL4 gene. Applicants respectfully disagree, nevertheless, claims 20 and 45 have been amended to provide a more clear nexus between steps a) and c) in claim 20 and clearly point out that the genomic DNA of claim 45 is from AXL4 gene (SEQ ID NO: 5). Applicant's amendment is not in acquiescence to the rejection, and Applicant expressly reserves the right to prosecute claims of similar or differing scope.

In conclusion, Applicants submit that in view of the detailed experimental protocol and the ample data provided in the specification, as described above, the skilled artisan would be enabled to carry out the method of the present claims. The Office Action asserts (page 9) that there is no predictability that claims 1-4, 7, 8, 10, 11, 20-39, 41-43, and 45 can be performed i.e., the art is unpredictable. However, Applicants submit that the level of skill in the art is high, as acknowledged by the Action (page 9). The methods of obtaining biological samples, extracting genomic DNA, and determining the methylation status of the same were well known in the art at the time of filing and are thoroughly described in the specification. Furthermore, Applicants have provided explicit disclosure of protocols for and the results obtained from comparison of human biological samples from subjects not having colon cell proliferative disorder and those with a variety of colon cell proliferative disorders. Thus, methods for detecting colon cell proliferative disorders in human subjects from biological samples by

analysis of methylation status and gene expression levels of ALX4 gene have been demonstrated in the specification. Accordingly, in view of the level of skill and the adequate disclosure, Applicants submit the specification as filed is fully enabling. In view of the amendments and the above discussion, reconsideration and withdrawal of the rejection are respectfully requested.

Rejection under 35 U.S.C. §112, 2nd Paragraph

Applicants respectfully traverse the rejection of claims 1, 2, 8, 10, 11, 21-39, 41-43, and 60 under 35 U.S.C. §112, second paragraph on the ground of alleged indefiniteness.

Specifically, the Office Action asserts that claims 1, 8, 21, and 45 are "vague and indefinite" based on the language contained in "step c)."

While not acquiescing to the rationale provided by the Office, and in order to further prosecution of the instant application, independent claims 1, 8, 21, and 45 have been amended accordingly to define Applicants' invention with greater clarity.

Claim 2 has been rejected as being allegedly vague and indefinite on the basis that it is "unclear that the CpG dinucleotide sequence in the claim means methylated CpG dinucleotide sequence or unmethylated CpG dinucleotide sequence." Applicants respectfully traverse and submit the CpG dinucleotide sequence recited in dependent claim 2 further limits the CpG dinucleotide sequence within ALX4 gene sequence (SEQ ID NO: 5) of claim 1 by requiring the sequence comprise at least 16 contiguous nucleotides of the ALX4 gene sequence (SEQ ID NO: 5) and not to limit the CpG dinucleotide to one that is either methylated or not mehtylated. Thus, it is not germane whether or not the CpG dinucleotide sequence of claim 2 is methylated.

Claim 21 has been rejected as vague and indefinite because "it is unclear how to correlate step b) of claim 20 with steps a) to d) of claim 21 and it is unclear that steps a) to d) of claim 21 is happened before or after which step of claim 20" and "it is unclear where a plurality of CpG dinucleotides is located." As claim 21 depends from claim 8, Applicants presume this is an inadvertent typographical error and submit that claim 21 has been amended to define Applicants invention with greater particularity. Support for the amendments to claim 21 may be found in

the specification as originally filed, for instance at page 27, lines 6-27 and page 11, line 24 bridging to page 12, line 25.

Claim 23 has been rejected as vague and indefinite on the basis of the term "method." Claim 23 has been amended to omit the term method and to define Applicants' invention with greater particularity.

Claim 26 has been rejected stating "the at least 9 nucleotides of b)" in the claim lack antecedent basis. As an initial matter, claim 26 depends from claim 21, which in turn depends from claim 8. Claim 8 recites the 9 contiguous nucleotides of claim 26. Notwithstanding, Applicants have amended claim 26 for further clarification.

Claims 28 and 29 have been rejected on the ground of an alleged lack of antecedent basis for "nucleic acid molecule or peptide nucleic acid molecule." Applicants have amended claims 28 and 29 to provide further clarification. Support for the amendments may be found, for instance, at page 34, lines 1-8 of the specification as originally filed.

Claims 41 and 42 have been rejected as being allegedly vague and indefinite stating "it is unclear that at least one detectably labeled nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID NOS: 312, 313, 428, and 429." As an initial matter, claim 42 does not recite "a detectably labeled nucleic acid molecule" but recites a nucleic acid molecule or a peptide nucleic acid molecule thus, Applicants presume this is an inadvertent typographical error in the Office Action. Applicants submit the language in claims 41 and 42 with regard to hybridization is clear: the detectably labeled nucleic acid molecule of claim 41 and the nucleic acid molecule or peptide nucleic acid molecule of claim 42 (all three of which comprise a sequence 9 nucleotides in length) hybridize to a sequence selected from the group consisting of SEQ ID NOS: 312, 313, 428, and 429.

In view of the foregoing discussion and amendments, Applicants respectfully request reconsideration and withdrawal of the rejection.

CONCLUSION

In view of the foregoing amendments and the remarks, it is submitted that the claims are in condition for allowance, and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this case.

The Commissioner is hereby authorized to charge the total amount of $470.00 to cover the payment of a One-Month Extension of Time fee ($65.00) and a Request for Continued Examination fee ($405.00), small entity. Applicants believe that no fee is deemed necessary with the filing of this paper. However, the Commissioner is authorized to charge any fees deemed necessary with the filing of this paper, or credit any overpayments, to Deposit Account No. 07-1896 referencing the above-identified docket number.

Respectfully submitted,

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